

Standard Guide for Microspectrophotometry and Color Measurement in Forensic Paint Analysis

Scientific Working Group for Materials Analysis (SWGMAT)

October 2007

Introduction

Color is one of the most important comparative characteristics of paints. The comparison of color is one of the first steps taken in a forensic paint comparison, and it is essential to note that this guide does not propose the use of instrumental color comparison for objects that are distinguishable to the eye. Since the 1940s, analytical instruments have been able to discriminate colors that the average human eye cannot distinguish. Microspectrophotometers (MSPs), in particular, allow for an objective measurement of the color of small, millimeter, or microscopic samples and are more precise or quantitative compared to the more subjective results of visual microscopical color comparisons.

Suitable instruments with appropriate optics, sensitivity, resolution, and dynamic range can measure and produce spectral curves of light energy from small samples as that light is transmitted, absorbed, or reflected by the sample. These spectra are collected over small measurement steps or increments of one-half to a few nanometers each. MSPs typically operate in the visible spectral region (approximately 380 to 800 nm) and also in the ultraviolet region (approximately 190 to 380 nm). They should not be confused with broadband or absorption filter-based, tristimulus systems or low-resolution, large-measurement-step (5 nm or more) spectral analyzers.

The spectral limits of different instruments can vary in all of the above-noted spectral regions and may also include the near-infrared region from about 780 to 2100 nm. The usefulness of this last spectral region in the analysis and comparison of paint fragments is currently indeterminate and will not be covered in this guideline.

Subjective terms such as *blue*, *violet*, or *purple* are inadequate descriptors of actual colors. MSP instruments can be used to describe or numerically “name” the color of an item by calculating the colorimetric values of the item. These values, or chromaticity coordinates, can be expressed in any one of several coordinate systems and are useful in the development and maintenance of color-comparison databases.

Colorimetric values are of limited use in actual color comparison of evidence samples because differing spectral curves can yield identical colorimetric values. This is commonly found in industrial or commercial paint-matching protocols where the principal consideration is to paint an item so it appears to be the same as others. The perception of the eye and the colorimetric values of two items may indicate that they are the same color, but the spectral curves of those items may still be distinguished. This leads to the use of the MSP in the comparison of visually indistinguishable colored items.

1. Scope

The following guideline is intended to assist individuals and laboratories that conduct forensic visible and ultraviolet spectral analyses on small fragments of paint. This MSP guideline deals primarily with color measurements within the visible spectral range but also includes some details concerning measurements in the UV range. It does not address other areas of color evaluation—such as paint surface texture or paint pigment particle size, shape, or dispersion within a paint film—which are evaluated by other forms of microscopy. Other MSP techniques—such as spectral luminescence,

fluorescence, and near-infrared (NIR)—are not included in this guideline because of their limited use, lack of validation, or lack of established efficacy in forensic paint analysis.

This guideline is directed at the color analysis of commercially prepared paints and coatings. It does not address the analysis or determination of provenance of artistic, historical, or restorative paints, but it may be useful in those fields.

This guideline does not purport to address safety concerns, if any, associated with its use. The user of this guideline has the responsibility for establishing appropriate safety and health practices and determining the applicability of regulatory limitations prior to its use.

2. Referenced Documents

2.1. ASTM International Standards

D16-03 *Standard Terminology for Paint, Related Coatings, Materials, and Applications*

D1535-06 *Standard Practice for Specifying Color by the Munsell System*

D2244-05 *Standard Practice for Calculation of Color Tolerances and Color Differences from Instrumentally Measured Color Coordinates*

E179-96(2003) *Standard Guide for Selection of Geometric Conditions for Measurement of Reflection and Transmission Properties of Materials*

E275-01 *Standard Practice for Describing and Measuring Performance of Ultraviolet, Visible, and Near-Infrared Spectrophotometers*

E284-06b *Standard Terminology of Appearance*

E308-06 *Standard Practice for Computing the Colors of Objects by Using the CIE System*

E805-06 *Standard Practice for Identification of Instrumental Methods of Color or Color-Difference Measurement of Materials*

E1610-02 *Standard Guide for Forensic Paint Analysis and Comparison*

2.2 ASTM. *Paint and Coating Testing Manual (Gardner-Sward Handbook. 14th ed.)*, J. V. Koleske, ed. ASTM, Philadelphia, Pennsylvania, 1995.

2.3 Scientific Working Group on Materials Analysis. Trace evidence quality assurance guidelines, *Forensic Science Communications* [Online]. (January 2000). Available: <http://www.fbi.gov/hq/lab/fsc/backissu/jan2000/swgmat.htm>.

3. Terminology

For definitions of paint-associated terminology used in the guide, see ASTM Standard D16-03, *Standard Terminology for Paint, Related Coatings, Materials, and Applications*; ASTM Standard E284-06b, *Standard Terminology of Appearance*; and ASTM Standard E1610-02, *Standard Guide for Forensic Paint Analysis and Comparison*.

Absorbance: The logarithm to the base 10 of the reciprocal of spectral internal transmittance. It is often expressed as a fraction or decimal value and may be referred to as optical density.

Absorption: A decrease in radiant energy when passing through matter, resulting in a corresponding increase in the energy of the absorbing system.

Bandwidth: Optical width of the monochromator exit slit or the optical width of a semiconductor detector pixel. This term also can refer to the wavelength interval over which the radiant energy of the selected wavelength peak is greater than 50 percent of the maximum peak intensity.

Chromaticity: The dimensions of a color stimulus, excluding luminous intensity and expressed in terms of hue and saturation or redness-greenness and yellowness-blueness. It is generally represented as a point in a constant luminance plane of a three-dimensional color space.

Chromaticity coordinates: The ratios of each of the three tristimulus values X, Y, and Z in relation to the sum of the three; designated as x, y, and z, respectively. They are sometimes referred to as the *trichromatic coefficients*. When written without subscripts, they are assumed to have been calculated for illuminant C and the 2° (1931) standard observer unless specified otherwise. If they have been obtained for other illuminants or observers, a subscript describing the observer or illuminant should be used. For example, x_{10D} and y_{10D} are chromaticity coordinates for the 10° observer and illuminant D. A “standard observer” refers to specific numerical values that represent the nominal color response of the human eye to different wavelengths of light. It is based on a study of the average retinal response of the human population.

Chromaticity diagram, Commission Internationale de l’Eclairage (CIE): A two-dimensional graph that describes a color as the locus of chromaticity coordinates on a field of monochromatic light varying from 380 to 780 nm in wavelength. X is the abscissa of the coordinate system, and Y is the ordinate. The diagram is used to plot and compare the colors of both luminous and nonluminous materials.

CIELAB unit E: (L*a*b*) Color Difference Equation from ASTM D2244: The color difference “E” between two samples is given by:

$$\Delta E_{CIE} (L^*, a^*, b^*) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where L* (pronounced “L star”) defines where a color falls on a vertical scale from light to dark, a* defines where the color falls on the continuum of colors between red and green, and b* defines where the color falls on the continuum of colors between blue and yellow.

Colorimetry: The converting of instrumental light measurements into psychophysical descriptions or numerical notations that can be correlated with visual evaluations of color and color differences.

Effect pigment: Any paint pigment that is designed to produce a significant change in color attribute(s) in a paint film when the film is viewed or illuminated from varied geometries.

Error ellipse (visual limits): The plotted limits of variation in visually indistinguishable color coordinate values that form an elliptical shape around a central color value when plotted in a chromaticity diagram.

Grating: A parallel set of linear, regularly repeating structures that, when illuminated, produce maxima and minima of light intensity as a consequence of interference. These maxima and minima vary in position with wavelength. This allows radiation of any given wavelength range to be isolated from a complex mixture of wavelengths and allows the grating to be used as a monochromator.

Illumination aperture: An element in the optical path of an MSP system that limits the area of illumination reaching the sample focal plane.

Measuring aperture: An element in the optical path of an MSP system that limits the area of illumination reaching the detector focal plane.

Monochromator: A device designed to isolate narrow wavelength ranges of light from complex, broad-spectrum radiation.

Photomultiplier tube (PMT): A photosensitive vacuum tube device that quantitatively converts photons of light into electrical energy.

Reflectance: The ratio of the intensity of reflected radiant energy to that of the incident radiant energy. In popular usage, it is considered the ratio of the intensity of reflected radiant energy from a test sample to that reflected from a defined reference standard.

Significant difference: A difference between two samples that demonstrates they did not originate from the same source.

Standard observer, CIE: The visual color perception data set adopted by the CIE to represent the response of the average human eye, when light-adapted, to an equal-energy spectrum. Unless otherwise specified, the term applies to the data adopted in 1931 for a 2° field of vision. The data adopted in 1964, sometimes called the 1964 observer, were obtained for a 10° field of vision and are generally used in industrial measurements.

Standard illuminant, CIE: Standard sources for which the CIE specified the spectral energy distribution as follows:

- Standard illuminant A, CIE: A tungsten filament lamp operated at a color temperature of 2856 K, approximating a blackbody operating at that temperature. It is defined in the wavelength range of 380 to 770 nm.
- Standard illuminant C, CIE: An approximation of overcast daylight having a correlated color temperature of approximately 6770 K. This color of light can be obtained by using a combination of illuminant A and a color-correcting filter. It is defined in the wavelength range of 380 to 770 nm.
- Standard illuminant D, CIE: An approximation of bright daylight illumination having a correlated color temperature of 6504 K in the spectral range of 300 to 830 nm. The UV portion of illuminant D, 300 to 380 nm, is necessary to correctly describe colors that contain fluorescent colorants or UV absorbers. The UV and visible portions are designated as UV_D and Vis_D . Illuminant D is based on actual measurements of the spectral distribution of daylight and is the most commonly used standard illuminant for spectral measurements.

Thermochromaticity: The characteristic of some materials, including some pigments, to change color as temperature changes.

Step width: Distance between two points of wavelength measurement in a spectrum.

Transmittance: A measure of radiant energy transmission through a material, in terms of the ratio of transmitted intensity to incident intensity (total transmittance). Both absorption and reflection influence total transmittance. The terms *transmittance* and *transmission* are used interchangeably in this document.

Tristimulus values, CIE: Amounts (in percent) of the three components in a three-color additive mixture required for matching a color. These components are designated as X, Y, and Z in the CIE system. The illuminant and standard observer color-matching functions must be designated. If they are not, an assumption is made that the reported values are for the 1931 observer (2° field) and illuminant C. The values obtained depend on the method of integration used, the nature of the sample surface, sample homogeneity, and instrument design. Tristimulus values are therefore not absolute values for a sample, but relative values dependent on the method used to obtain them. Examples of calculations of tristimulus values in the CIE system can be found in ASTM E308-06.

Wood's anomaly: The effect caused by a polarization of diffracted incident energy due to nonuniform reflection as the angle of incidence varies on a holographically blazed monochromator. This effect can be observed as a distinct transmittance maximum within the spectrum.

4. Summary of Practice

4.1. One of the most obvious decorative properties of paint is its color. Usually one or more major pigments are used in a paint, together with one or more minor pigments whose purpose is to modify color or other film properties. The interaction of pigments and light is very complex. Light can be scattered, absorbed, and reflected by pigments within the paint layer.

4.2. The processes responsible for producing the color of pigments include:

- Crystal-field effects with transition metal compounds (most inorganic pigments belong to this group).
- Electron transitions between molecular orbitals in compounds with conjugated double bonds (organic pigments).
- Electron transitions in compounds with conduction energy bands (metal pigments and pure semiconductors).
- Other processes caused by geometrical and wave optics (interference, scattering, diffraction in optically variable pigments, liquid-crystal pigments, etc.).

All of these processes are based on the interaction between UV-VIS light waves themselves or with electrons in pigments and can be measured and compared using MSP.

4.3. The analysis of paint using MSP can be carried out using either transmission or reflectance techniques. However, one should be aware that paint color comparisons and pigment identifications using reflectance measurements are difficult tasks because of the effects of surface observation angle, surface texture, and sample curvature. This method is seldom used for detailed color comparison or pigment identification, but it can be useful in exclusionary comparisons of bulk colors.

4.4. Basic Principles

4.4.1. An MSP can measure reflected or transmitted radiation at selected wavelengths. The resulting total spectral curve of a colored sample is produced by comparing the light reflected from a sample to the light reflected from a white reflectance standard or by comparing light transmitted by the sample to the light transmitted by a colorless background.

4.4.2. Paint colors are usually measured in transmission through thin sections. Reflectance techniques also can be used, especially when fluorescence measurements in the UV range are desired. When performing measurements on UV-absorbing materials in the UV region, transmission techniques are recommended.

4.4.3. In reflectance measurements of paint color, a sample is illuminated, and the fraction of light reflected in the visible range is recorded. The reflectance curve obtained provides a representation of the color. In transmission measurements, a thin section of paint is illuminated, and the fraction of light transmitted or absorbed in the visible and/or UV range is recorded.

4.4.4. Transmission spectra may be plotted in either percent transmittance or absorbance. Reflectance spectra may be plotted in either percent reflectance or absorbance.

4.5. Colorimetry

4.5.1. In order to achieve accurate color values, a number of instrumental parameters have to be defined. In 1931, the CIE defined standard illuminants, measuring conditions, and the three standard primary colors: [X], [Y], and [Z]. The amount of each of these colors needed to match a specific color

can be calculated. These calculations yield a mathematical representation of a color (tristimulus values).

4.5.2. Using a mathematical equation, these tristimulus values can be converted into L^* , a^* , and b^* coordinates. The Colour Measurement Committee of the Society of Dyers and Colourists recommends this system for the description of color. From the values of L^* , a^* , and b^* , color differences can be obtained in CIELAB units. Some of these issues are presented in ASTM E805.

4.6. Metamerism

4.6.1. Two samples can appear to have the same color under one type of illumination but may appear dissimilar under different lighting conditions. This phenomenon is one form of metamerism. A second form of metamerism occurs when two samples appear to be the same color under all lighting conditions, yet their reflectance or transmittance curves are different. Both forms of metamerism are due to differences in pigment compositions. It is important to note that two objects with different reflectance or transmittance curves can have identical colorimetric values (tristimulus values or chromaticity coordinates). Therefore, it is not recommended to use colorimetric values alone for detailed sample comparisons.

4.7. Instrumentation

4.7.1. The MSP has been designed to meet the requirements of analyzing the color of a sample *in situ*. In scanning spectroscopy, an MSP consists basically of a microscope with a stabilized light source, a spectral dispersive element, a detector, and a data processing device. The main function of the microscope is to reflect or transmit light energy efficiently, uniformly, and reproducibly. All MSPs are single-beam instruments; a standard or a blank is measured, the result is stored, and a sample is measured and ratioed to the blank to yield a transmittance or reflectance spectrum. MSP systems are generally used in forensic analysis because of the small sample sizes presented by paint film fragments, but instruments for color measurement from large samples (spectrometers) also can be used for compiling color databases. Large-area measurements can differ from microscale measurements because of the effects of averaging. It can be difficult to compare data from an MSP to that of a macroscale spectrometer.

4.7.2. A monochromator produces monochromatic light of a certain spectral bandwidth. The sample is scanned step by step, and a spectrum is created point by point. This type of instrument is known as a scanning spectrometer and generally uses a PMT detector. However, in a multichannel spectrometer, all spectral regions are detected simultaneously and a semiconductor detector is commonly used.

4.7.3. The use of semiconductor detectors is relatively new in forensic analysis. Spectrometers using these devices are composed of a diffraction grating together with an array detector that acts as the photosensitive device. The resolution of the detector will depend on the number of diodes or other detector elements in the array, the dispersion and line spacing of the grating, and the distance between the grating and the array. Measurement time is drastically reduced compared to PMT systems because of the simultaneous detection of the full spectral range.

4.7.4. Commercially available MSPs have incorporated the requirements of selecting and controlling operational settings, data collection, data processing, and data plotting into a computerized system. Accordingly, all MSPs use software packages to cope with these tasks.

5. Significance of Use

5.1. The color of paint samples can be measured by reflectance (visible range) or transmission (UV-visible) for comparison purposes. Transmission measurements, rather than reflectance measurements, are used for the analysis of UV absorbers in clearcoats, the classification of

pigments, and the detailed analysis of effect pigments that are not opaque.

5.2. This guide is designed to assist an analyst in the selection of appropriate sample-preparation methods and instrumental parameters for the analysis, comparison, or identification of paint pigments and colors.

5.3. It is not the intention of this guide to present comprehensive theories and methods of microspectrophotometry. The analyst must have an understanding of UV-visible microspectrophotometry and the general concepts of specimen preparation prior to using this guide. This information is available from manufacturers' reference materials, training courses, and references, such as Eyring (2001) and Stoecklein (2000).

6. Analytical Procedures

6.1. Reflectance Test Specimens

6.1.1. Surface reflectance measurements should be carried out on clean and undamaged sample areas. The surface of the sample should be cleaned carefully with alcohol or another suitable solvent to make it dust-free. Measurements are usually conducted without further alteration of the sample surface so that surface features that might distinguish between samples are not changed. In some cases, such as in the determination of the origin of a vehicle in a hit-and-run case, it is necessary to remove surface features. Weathered surfaces can be removed by polishing the paint using a diamond paste or another polishing medium so the measured properties of the surface are comparable to data collected from new, undamaged paint surfaces.

6.1.2. Diffuse reflectance spectra change dramatically as the surface finish of the sample varies (Eyring 2001). In order to ensure reproducibility, the pigments in the sample should be the limiting factor in the smoothness of the surface. Hence reflectance measurements from edge-mounted samples require that the paint edge be carefully polished to a surface finish approaching the size of the smallest pigment particles in the paint layer(s). Usually, 0.25-micrometer polishing grit is sufficient. Samples for comparison should be prepared in the same manner. Reproducing the surface finish may be simplified by mounting and polishing edge-mounted known and questioned samples side by side.

6.1.3. In order to obtain optimal results during color measurements, the surface must be free of scratches, dirt, and blemishes and must be mounted normal to the optical axis of the microscope. The surface of the paint sample must be in sharp focus. The area measured should be as large as possible in order to average any heterogeneity present. This is particularly important for paints containing effect pigments.

6.1.4. Paint samples that are very small or difficult to handle should be embedded in a resin so that a cross section of the paint can be examined. Before measurement, the embedded sample should be exposed and, when necessary, polished. The MSP measuring field must fall within the boundaries of the paint layer being analyzed in order to avoid interference from adjacent layers.

6.1.5. Reflectance measurements are not recommended with very small samples of effect paints because their limited areas do not allow for multiple measurements that would provide a representative spectral response curve. In this case, thin cross sections should be prepared for analysis by transmission methods.

6.1.6. Smear paints are unsuitable for reflectance measurements but may be analyzed by transmission methods. See Section 6.2.2.

6.2. Transmission Test Specimens

6.2.1. For transmission measurements, thin sections should be prepared and examined using VIS or UV-VIS spectroscopy. The MSP measuring field must fall within the boundaries of the paint layer being analyzed in order to avoid interference from adjacent layers. The area measured should be as large as possible in order to average any heterogeneity present.

6.2.2. Transmission measurements may be conducted on paint smears. This analysis may include an overall area within the smear or individual pigment particles. If the smeared paint is a mixture, different portions of the smear can be separated and mounted for analysis. These results can provide conclusive exclusionary data but may not provide meaningful associative information.

6.2.3. Measurements in the UV range require quartz or mirror optics, quartz slides and coverslips, and a nonfluorescing, UV-transparent mounting medium such as glycerol.

6.2.4. Paint sections should be approximately 3 micrometers thick for UV-VIS measurements of pigmented layers. Because of the low concentration of UV-absorbers in clearcoats, approximately 20-micrometer-thick sections should be prepared for analysis. To prevent discrepancies in measurements caused by differences in section thickness, the questioned and known samples should be embedded side by side and sectioned together.

6.2.5. Photometric (quantitative) reproducibility may be difficult to achieve when thin peels of paint layers and hand-cut cross sections are used for analysis. This may not be problematic where spectral curves exhibit exclusionary features, but it can lead to difficulties in interpretation of shade differences.

6.2.6. During UV analyses, a system with a monochromator located in the illumination beam path ahead of the specimen will protect the specimen against overheating and UV degradation. Semiconductor detector systems typically do not have this problem because they have short measurement times.

6.2.7. A high-magnification, oil-immersion objective (about 100X) and condenser are usually necessary for the measurement of small individual pigment particles or particles within pigment aggregates. Dry 100X objectives are available, but their resolution and light-gathering power may be too low for some measurements.

6.2.8. Pigment classification by color analysis usually requires that a spectral library be prepared on the instrument in use. Second-party libraries are suitable for preliminary identifications or classifications. It is recommended that a known sample of the suspected pigment be analyzed before making an identification.

6.3. General Recommendations

6.3.1. To demonstrate the range of variation present in a paint sample, it is suggested that at least 5 spectra be collected from each noneffect paint film and up to 10 spectra for effect finishes, each representing different areas of the sample. This number can be adjusted according to circumstances and is dependent on the degree of spectral consistency observed. Small sample size or poor sample condition can preclude the acquisition of sufficient spectra to meet these criteria.

6.3.2. A general discussion of geometric considerations for reflectance and transmission measurements is found in ASTM Standard E179-96(2003), *Standard Guide for Selection of Geometric Conditions for Measurement of Reflection and Transmission Properties of Materials*.

7. Instrument Calibration

7.1. Calibration ensures that an instrument is operating within required parameters and that any errors that might affect data or analytical conclusions are known, limited, accounted for, and/or

corrected. It is necessary to demonstrate that the instrument is calibrated and to maintain a record of the calibration results.

7.2. To ensure that historical calibration data are comparable, the optical system should be optimized each time the system is calibrated. If possible, Köhler illumination should be used.

7.3. Normal operating parameters should be defined, and the entire MSP system should be allowed to warm up and stabilize according to the manufacturer's instructions or laboratory experience, whichever is longer. Calibration should not take place until this condition is met.

7.4. It is essential to demonstrate wavelength and absorbance/photometric accuracy by calibration. If colorimetry is used as a comparative feature, then that too should be calibrated using National Institute for Standards and Testing (NIST) or other traceable standards. Some other sources of traceable standards include Hemmendinger Color Laboratory, Princeton, New Jersey; Labsphere, Inc., North Sutton, New Hampshire; Oriel-Spectra Physics, Mountain View, California; and Starna, Atascadero, California. Their current addresses and contact numbers can be found by Internet search.

7.5. Wavelength accuracy over the UV-visible range should be checked routinely with the aid of NIST-traceable holmium, erbium, or didymium oxide filters, using the working instrument resolution. It is also recommended that wavelength accuracy be checked periodically at the highest resolution of the instrument. These measurements must be carried out in transmission. Because exact positions of the absorption bands vary from one batch to another, it is important that the filters be traceable. Resolution also can be checked routinely using these filters.

7.6. Traceable neutral-density filters should be used to demonstrate the photometric accuracy of the system. It is especially important for colorimetric measurement to demonstrate that the photometric response of the instrument is linear. Köhler illumination should be set up before calibration checks are done. A typical set of neutral-density calibration filters might include filters of 0.1, 0.5, 1.0, 2.0, 2.5, and 3.0 absorbance units. Quartz filters must be used for measurements in the UV range.

7.7. Instrument long- and short-term stability and consistency may be demonstrated using the 100 percent transmittance line by ratioing two spectra of a clear and colorless slide, mountant, and coverslip preparation. The 100 percent line also can be evaluated in reflectance by measuring a white standard such as BaSO₄ or MgO or Opal glass MS 20 (Hemmendinger Color Laboratory).

7.8. Suitable color standards, such as those produced by Labsphere, may be used for checking the overall operation of the equipment and the colorimetry calculation program of the software.

7.9. All measurements should be carried out under similar conditions. These conditions should include the length of time that a sample resides in the illumination beam. Sample temperature can increase with examination time because the sample absorbs heat from the light beam and the thermochromaticity of some pigments may result in distinct color differences with temperature changes of a few degrees.

7.10. The generally used illumination and measuring geometry in reflectance analysis of 45°/0° can be best achieved with the help of a dark-field/bright-field illuminator fitted with dark-field objectives. Some older systems may include two objectives, one for reflection illumination and another for diffuse reflectance measurement, to satisfy this geometry.

7.11. Background, system, or reference transmittance spectra should include the absorbance contribution of all system components except the sample of interest. These components include the microscope slide, coverslip, and mounting medium. The resulting spectra can be used to monitor illuminator performance and warn of unsuitable system adjustments remaining from prior analyses.

7.12. Ratioing two successive background spectra yields a 100 percent line. The transmittance at all

wavelength steps will usually be about 100 percent +/- 2 percent. If a greater deviation from the 100 percent line is observed, it may indicate an instability of the illumination system (especially a xenon lamp) or it could indicate the need for slower scanning speed or longer spectral integration times. The 100 percent line also offers documentation of the basic noise-level limits and operational limits of the system.

7.13. It is recommended that calibration be conducted at least once a month or just prior to analysis if used less frequently. Calibration should also be performed after any instrument maintenance. Calibration procedures and results generally need to be recorded for historical reference. A historical record of this data may not only offset court challenges but also may provide an operator with an early warning of system deterioration.

8. Instrument Setup and Scanning Parameters

8.1. Measurement ranges may be adjusted as necessary. The wavelength range selection should reflect the maximum amount of information needed for a particular examination. Depending on the application and the instrument used, it will be approximately in the ranges of 200 to 780 nm (transmission UV-VIS), 380 to 780 nm (VIS reflectance and colorimetry), and 240 to 400 nm (transmission UV). The actual range(s) selected will vary with instrument design, noise-level limits, and the dynamic range of the detector.

8.2. The resolution of some instruments can be adjusted. Resolution usually is set higher for transmission measurements (i.e., 2.5 nm or less) than for reflectance measurements (i.e., approximately 10 nm). This is due to the generally narrower spectral features present in transmittance spectra. High-resolution scans can be used for both types of measurements if desired, but signal-to-noise ratios may deteriorate to unacceptable levels at unnecessarily high resolution settings.

8.3. Illumination field, measuring aperture, and magnification must be adapted to the sample under investigation.

8.3.1. The measuring aperture should be set as large as possible for a given sample and centered on the optical axis of the spectrometer system to improve signal-to-noise ratio and reduce the possible effects of sample heterogeneity.

8.3.2. When possible, the illumination aperture (field aperture) should be set to fall inside the measuring aperture field of view to increase the signal-to-noise ratio and improve photometric precision.

8.3.3. A 20X to 40X objective may be necessary for effective transmission measurements, and a 100X objective can be used for measurements involving very small particles and pigment particle identification.

8.4. In transmission, the areas selected for analysis should be as homogeneous as possible and should be uniform in size for the questioned and known samples. Large particles such as effect pigments should be avoided. It is also necessary that the thin section of the sample be as flat as possible to avoid stray (off-axis) light effects.

8.5. Sample areas selected for reflectance analysis should be uniform in size and free of dust and scratches for the questioned and known samples.

8.6. The reflectance background reference scan must be made using a white standard (e.g., BaSO₄, MgO, Opal glass MS20). The reflectance background scan should be acquired prior to scanning samples. It is recommended that a new reflectance background spectrum be measured for each new reflectance sample. This recommendation is a precaution to avoid spectral errors caused by

short-term instrument drift.

8.7. For transmission measurements, it is necessary to run a new background reference scan for each new microscope slide and coverslip preparation and for each new aperture configuration.

8.8. The transmission or reflectance background and sample scans must be made using all of the same parameters (i.e., objective(s), aperture area(s), lamp voltage, scan or spectrum averaging, step width, resolution, or sample focal plane).

9. Spectral Comparison and Interpretation

9.1. It is worth repeating the general caution that for comparative color examinations, a basic rule must be complied with: The known sample(s) must originate from an area as near as possible to the suspected source point of the questioned sample(s). Differences may arise in top-surface reflectance measurements of paint samples from the same vehicle or other painted surfaces because of differences in sunlight exposure, weathering conditions, or repaired areas. Transmission measurements also can exhibit variations for the same reasons.

9.2. Spectra can be compared by overlaying them on a light box or by plotting them on the same graph. Mean-value spectra, generated from several scans of each sample and bracketed by curves showing +/- one or more standard deviations of the mean sets, also are suitable for comparison. Three standard deviation units will cover approximately 99 percent of the expected sample variation under normal distribution conditions.

9.3. Spectra can be displayed and compared in percent reflectance or percent transmittance and absorbance formats. Some spectral features can be seen only in one or the other display format. For example, when comparison of percent transmittance spectra does not discriminate between those spectra, the spectra also should be compared in absorbance format, which may provide additional information. Therefore, spectra should be compared in both formats. In accordance with Beer's law, only absorbance spectra yield a linear variation with the concentration of the analyte. If questioned and known samples contain more than one component and therefore more than one absorption band, the absorbance spectra then provide relative quantitative data about the concentration of the components. Spectra with single absorption bands that are displayed in absorbance can provide relative quantitative data about the concentration of a component in different samples of the same thickness.

9.4. Ideally, known and questioned spectra should be identical if the samples are to be considered indistinguishable. In practice, known or questioned sample spectra may exhibit shape variations over different regions of the sample. If no questioned sample spectra fall completely within the range of variation exhibited by the spectra of known samples, the known sample can be eliminated as a potential source.

9.5. Fundamental Steps of Comparison and Interpretation

9.5.1. The analyst should gain familiarity with, as well as document, the absorption range and variety of curve shapes expressed in known samples by studying them thoroughly in both percent transmittance and absorbance formats. Standard-deviation curves can prove particularly useful in these instances for estimating the sample variation range of the known. First and second derivative functions of the spectra also can assist in identifying inflection points and help in the discrimination of metameric samples. Effective use of derivative functions requires that spectra have high signal-to-noise ratios.

9.5.2. The known and questioned sample comparison begins with the examination of the whole spectrum, followed by critical examination of each specific peak. The comparison should include examination of peak shape, minima, maxima, inflection points, troughs, shoulders, and the curves or

slopes between peaks.

9.5.3. Questioned absorbance spectra should overlie uniformly within the range exhibited by the known sample, if the known is to be considered a possible source of the questioned sample. The analyst can expect the absorbance spectra to have identical shapes, although they might not superimpose exactly.

9.5.4. If paint samples are measured in reflectance and both samples are not oriented perpendicular to the optical axis of the microscope, variations may be observed in their spectra. The reflectance differences due to sample orientation are generally seen as variations in reflectance intensity rather than in curve slopes or absorbance cutoff points. Multiple sampling locations may resolve these variations. With some samples, variations in spectra will be minimized if the samples are embedded side by side and smoothed by cutting or polishing.

10. Colorimetry Values Comparison and Interpretation

10.1. Colorimetry can be used for preliminary sample comparisons, but its main application is in the automatic searching of databases. An automotive paint database containing colorimetry values can yield information concerning the possible makes, models, and manufacturing years of vehicles involved in hit-and-run cases.

10.2. Samples of paint from areas next to each other on new vehicles with original paint will generally yield color differences of between 1 and 3 CIELAB units (Cousins 1989). Nevertheless, these values can be higher, especially for effect paints and neutral colors or in situations where the measuring area is small (e.g., cross-sectioned samples).

10.3. Comparisons can be carried out on mean chromaticity values. It is also important to compare the ranges of chromaticity values between the questioned and known samples. When the range of chromaticity values is greater in one sample than the other, it may be an indication that the questioned sample did not originate from the same source as the known sample, even in situations where the mean values are the same.

10.4. The analyst should not rely solely on colorimetry results when making comparisons. A spectral comparison must always be undertaken because different curves, such as those from metameric samples, can yield similar chromaticity coordinates.

10.5. Error ellipses should not be used as evaluation tools for comparisons because they can vary dramatically with sample size, heterogeneity, and the presence of effect pigments.

11. Conclusions

The results of paint comparisons performed by MSP are typically expressed as one of three general concepts: indistinguishable, distinguishable, or inconclusive. It is important to understand that these results refer only to the comparison of MSP spectra and not to a complete paint examination.

- *Indistinguishable:* When comparative analyses do not demonstrate significant differences between the samples, a statement can be made that no differences were indicated within the discriminatory limits of the analytical method.
- *Distinguishable:* When comparative analyses demonstrate one or more significant differences between samples, a statement of dissimilarity can be made.
- *Inconclusive:* The comparative analyses of some samples, such as damaged and/or contaminated samples, may yield results in which the differences may or may not be

significant. Therefore, no definitive conclusion can be drawn.

12. Databases

12.1. Some agencies have created their own databases that are specific for automotive paint topcoats or color coats. Their intended use is to assist in identifying a certain color, and thus a possible make of vehicle, by comparing measured chromaticity coordinates with the data from known samples. It is common for these databases to be compiled using a commercial or industrial reflectance spectrophotometer with large measuring fields of up to 5 cm². Samples that appear homogeneous using the large measuring areas of these instruments may be heterogeneous at the microscopical measurement level. Variations in topcoat color, and the fact that questioned samples generally are measured with a different instrument than that used to produce the database, can lead to erroneous associations and missed associations. Therefore, in order to compare samples measured with microspectrophotometers to a database compiled using these commercial or industrial instruments, mathematical corrections must be used.

12.2. MSP-to-database comparisons should be used only as a complementary technique to other analytical methods.

13. Documentation

Subject to a laboratory's procedures, case notes should include:

- All of the instrumental data used to reach a conclusion.
- A unique sample designation.
- A unique laboratory number.
- A description of the evidence analyzed by MSP.
- A description of the sample preparation.
- Analytical instrumentation used.
- Operating parameters.

All data must include the operator's name or initials and the date of analysis. Refer to SWGMAT *Trace Evidence Quality Assurance Guidelines* for further information.

14. References

Adolf, F. P. and Dunlop, J. Microspectrophotometry/colour measurement. In: *Forensic Examination of Fibres*. J. Robertson and M. Grieve, eds. Taylor & Francis, London, England, 1999.

Billmeyer, F. W. Jr. and Saltzman, M. *Principles of Color Technology*. 2nd ed., John Wiley & Sons, New York, 1981.

Caddy, B. *Forensic Examination of Glass and Paint: Analysis and Interpretation*. Taylor & Francis, London, England, 2001.

Cassista, A. R. and Sandercock, P. M. L. Comparison and identification of automotive topcoats: Microchemical spot tests, microspectrophotometry, pyrolysis-gas chromatography, and diamond

anvil cell FTIR, *Canadian Society of Forensic Science Journal* (1994) 27:209–223.

Commission Internationale de l'Eclairage. Official recommendations of the International Commission of Illumination, *CIE Technical Publication no. 15*, Bureau Central de la CIE, Paris, France, 1971.

Cousins, D. R., Holding, R. H., Locke, J., and Wilkinson, J. M. A data collection of vehicle topcoat colours. 4. A trial to assess the effectiveness of colour identification, *Forensic Science International* (1989) 43:183–197.

Cousins, D. R., Platoni, C. R., and Russell, L. W. The variation in the colour of paint on individual vehicles, *Forensic Science International* (1984) 24:197–208.

Eyring, M. Visible microscopical spectrophotometry in the forensic sciences. In: *Forensic Science Handbook, Vol. I*. 2nd ed., R. Saferstein, ed. Prentice-Hall, Englewood Cliffs, New Jersey, 2001.

Johnston-Feeler, R. and LeSota, S. *Glossary of Color Terms*. Federation of Societies for Coatings Technology, Blue Bell, Pennsylvania, 1981.

McLaren, K. *The Color Science of Dyes and Pigments*. 2nd ed., Adam Hilger, Bristol, England, 1986.

Nowicki, J. and Patten, R. Examination of U.S. automotive paints: I. Make and model determination of hit-and-run vehicles by reflectance microspectrophotometry, *Journal of Forensic Sciences* (1986) 31:464–470.

Stoecklein, W. The role of colour and microscopic techniques for the characterization of paint fragments. In: *Forensic Examination of Glass and Paint: Analysis and Interpretation*. B. Caddy, ed. Taylor & Francis, London, England, 2001.

Terstiege, H. Color tolerances in automotive paints, *Journal of Coatings Technology* (1986) 58 (735):37–41.

Walsh, K. A. J., Axon, B. W., and Buckleton, J. S. New Zealand bodyfillers: Discrimination using IR spectroscopy, visible microspectrophotometry, density and SEM-EDAX, *Forensic Science International* (1986) 32:193–204.